Application of Genome-scale Models to the Optimization of Recombinant Protein Production in *Escherichia coli*

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Escherichia coli has been the organism of choice for the production of many recombinant proteins with high therapeutic value. However, while there have been several studies covering the recombinant protein production process, including analysis of the transcriptome, proteome, fluxome or metabolome, there has been a lack of integrative approaches able to combine genomic and physiological information about those processes with high-throughput analysis.

In this work, a systems biology approach for modeling recombinant protein production processes was used aiming its optimization.

The existing genome-scale metabolic model of *E. coli* [1] was modified to represent the recombinant protein production process, by including an additional equation based on the protein's amino acids content. Additionally, equations that represent the metabolic burden caused by the presence of plasmids were added, based on knowledge about the precursor balances and energetic requirements for plasmid replication and marker protein expression. This modified metabolic model was used on simulations using the FBA approach [2]. A knockout algorithm [3] was then used to identify possible gene deletion strategies that favor recombinant protein production. The results were interpreted using a developed computational tool that allows the visualization of the metabolic model together with different types of genome-scale data.

For the validation of the genome-scale model in high-cell density processes, highly reproducible fed-batch fermentations were run with constant specific growth rate. A developed data acquisition and control system allows to control the substrate addition rate, and to acquire on-line the fermenter' s weight, to calculate oxygen and carbon dioxide transfer rates, as well as to obtain glucose and acetate concentrations using a developed Flow Injection Analysis system.

1. Reed, L. *et al*. (2003) An expanded genome-scale model of *Escherichia coli* K-12 (iJR904 GSM/GPR). *Genome Biol.*, 4: R54.
2. Edwards, J. S., Covert, M., Palsson, B. (2002) Metabolic modelling of microbes: the flux-balance approach. *Environ. Microbiol.*, 4: 133-140.
3. Patil, K. R. et *al*. (2005) Evolutionary programming as a platform for *in silico* metabolic engineering. *BMC Bioinformatics* 6:308.